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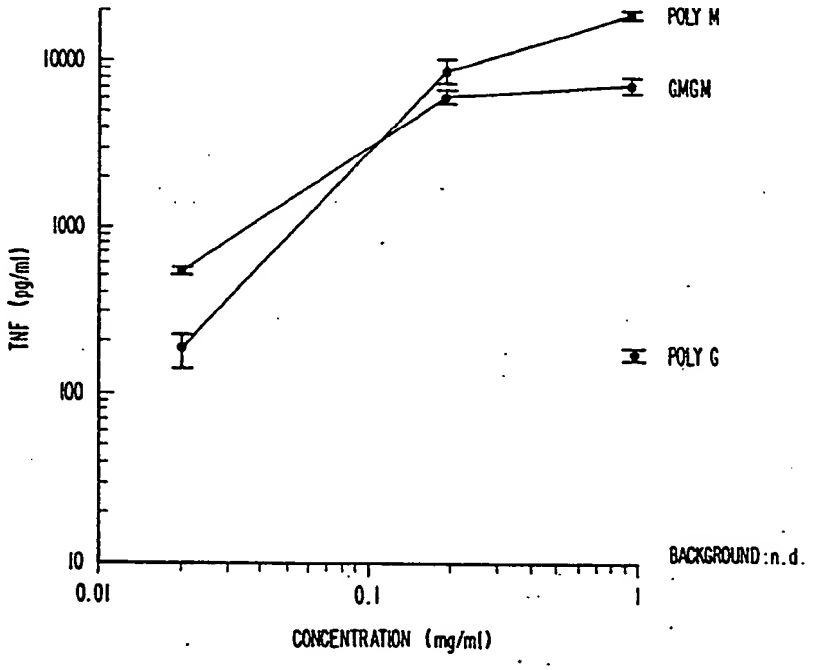

(57) Abstract

A composition which induces the release of cytokines, and is therefore useful in wound healing and treatment of tumors is disclosed. The composition comprises biopolymers such as alginate comprised of at least 70 % molar β-D-mannuronic acid and β-D-mannuronate. The method of making such compositions and use of the same are also disclosed.

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DESCRIPTION

Mannuronic Acid Containing Alginate Wound Healing Composition and Method

This is a continuation-in-part of of United States Serial No. 07/468,905 filed January 23, 1990.

Background of the Invention

Field of the Invention

This invention relates to the fields of biochemistry and medicine and more particularly to the field of wound healing.

Art Background

Alginate is a heterogeneous group of linear binary copolymers of 1-4 linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G) or combinations of the foregoing (GMGM). The monomers are arranged in a block-wise pattern along the polymeric chain where homopolymeric regions are interspaced with sequences containing both monomers. The proportion and sequential arrangement of the uronic acids in alginate depend upon the species of algae and the kind of algal tissue from which the material is prepared. Various properties of different types of alginates are based upon the guluronic acid makeup of the particular alginate. For example, viscosity depends mainly upon the molecular size, whereas the affinity for divalent ions essential for the gel-forming properties are related to the guluronic acid content. Specifically, two consecutive di-axially linked G residues provide binding sites for calcium ions and long sequences of such sites form cross-links with similar sequences in other alginate molecules, giving rise to gel networks.

Commercial alginates are produced mainly from Laminaria hyperborea stem (30% M), Laminaria hyperborea

leaf (55% M), Macrocystis pyrifera (60% M), Laminaria digitata (55% M), Ascophyllum nodosum (65% M), Laminaria japonica (65% M), Ecklonia maxima (55% M), and Lessonia negrescens (60% M). These alginates are all relatively low in M content, with the highest being only 65%.

Additionally, alginates may be obtained by isolation and purification techniques from certain bacteria. *Azotobacteria vinelandii* produces O-acetylated alginate with a content of L-guluronic acid ranging from 15% to 90%. *Pseudomonas aeruginose* under certain growth conditions produces poly-mannuronic acid and such bacteria as well as certain other alginate producing Pseudomonads are not able to produce polymers containing G-blocks. Other Pseudomonas such as *P. putida*, *P. mendocina*, *P. fluorescans* and *P. syringae* are also known as producers of alginate comprising a high M content in the range of 95-100% M.

A few algae sources are capable of producing alginate having a G content of less than 30%. Such algae include Durvillea and Ascophyllum. However, bacteria are the preferred source of high M containing alginate. Alginates having high or low contents of G or M residues may be obtained from specific portions of the algal tissue, such as, alginate having a high content of guluronic acid may be obtained from the outer cortex of old stipes of *L. hyperborea*, and alginate very rich in mannuronic acid residues may be obtained from the vegetative growth zone in the fronds of *L. hyperborea* or from the fruiting bodies of *Ascophyllum nodosum* and *Fucus vesiculosus*. In the latter case almost pure polymannuronic can be obtained. Alginate having a high content of guluronic acid can also be prepared by chemical fractionation or by enzymatic modification in vitro using mannuronan C-5 epimerase. This enzyme is able to introduce G-blocks into an existing alginate polymer, producing polymers with high G-block content.

A number of materials have been found to enhance or induce naturally occurring fibrosis or fibroblast

formation. L-lysine is one such material, and fibrinogen is another. Such materials, if made into an appropriate composition, may be used as a coating or treatment for injuries and may cause increased healing speeds as a result of the induction of fibrosis around the injured area to which a fibrosis inducing agent is applied.

However, alginate has not heretofore been shown to be effective as an inducer of proliferation of fibroblasts. Michaelis (U.S. Patent No. 4,837,024, hereafter the '024 patent) described chemotaxis of fibroblasts produced by small amounts of alginate, but stated that increased quantities of the alginate decreased the amount of fibroblasts attracted to the area. Additionally, Michaelis teaches nothing about the effects of the composition of alginate on chemotaxis, or any effects on the fibroblasts other than chemotaxis.

Several factors, such as cytokines and growth factors, play an important role in the wound healing process. These include the growth factors epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and transforming growth factor-beta (TGF-β). In addition to these growth factors, cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF) and interleukin-6 (IL-6) also play a role in the wound healing process. These factors work in various ways in combination with other materials to induce proliferation of dermal and epidermal components such as fibroblasts.

Prior to this invention, it was known that the administration of certain cytokines such as IL-2 can enhance a body's immune response to tumor antigens presented by tumor cells. (Rosenberg et al, (1985) J. Exp. Med. 161:1169; Rosenberg et al., (1988) Ann Intern Med, 108:853; Pizza et al., (1988) Lymphokine Res., 7:45). The administration of these molecules resulted in decreased presence of tumor cells in the patient due to the enhanced immune response. The cytokines used in such

experiments were either purified from blood fractions or generated through genetic engineering.

Before this invention, there was no teaching of the incorporation of growth factors or cytokines in wound dressings to promote healing. Luck et al. (U.S. Patent No. 4,619,913, issued October 28, 1986) (the '913 patent) discusses the use of cytotoxic factors incorporated into a matrix to be applied to tumors to kill the tumor tissue. The '913 patent describes the incorporation of such materials as radioactive pellets, repair inhibitors, and immunomodifiers such as interferons, lymphokines and tumor growth factor-β to either directly kill the cells or induce the body to react against and remove the tumorous cells. For example, as discussed above, immunomodifiers such as interferons are known to stimulate the body's immune reaction against tumor cells. The use of lymphokines and growth factors in this context does not induce healing of lesions, but rather induces death of unwanted tissue from the body.

Summary of the Invention

The present invention demonstrates the unique biological activity of mannuronic acid moieties in cytokine induction, useful for such diverse purposes as wound healing and treatment of tumors.

Thus, the present invention comprises a biopolymer such as alginate or alginic acid which, when applied to a wound of a mammal, induces the release of cytokines as well as fibrosis, and which may be formulated as a wound dressing, thus improving wound healing. Furthermore, growth factors may be incorporated into the invented material which would further enhance the results of the present invention.

The composition comprises a biopolymer composed of at least 70% β-D-mannur nic acid or mannuronate moieties (hereinafter referred to as at least 70% M). One example of such a polymer is alginate comprising at least 70% M,

with the remainder its C-5 epimer α-L-guluronic acid (G). Other biopolymers, such as oxodized guar gum or oxidized mannan, are also useful if they contain at least 70% M. Methods of making and using the invented compositions are also disclosed. Alginate derived from *Azotobacter vinelandii*, *Pseudomonas aeruginosa*, *P. putida*, *P. mendocina*, *P. fluorescens* and *P. syringae* are preferred as starting material for alginate having a high M content.

Such biopolymers are useful in treating internal as well as external wounds, through stimulation of cytokine release and the resulting fibrosis. Fibroblasts and other cells important in wound healing are induced to proliferate and heal the wound. Thus, methods and compositions for healing wounds are provided by this invention.

The compositions of this invention can be applied topically to external or internal wounds, or can be taken orally to treat internal wounds.

It is yet another object of the present invention to provide a composition which induces cytokine release, thereby promoting fibroblast formation and enhancing wound healing and angiogenesis.

It is another object of this invention to provide compositions for treatment of tumors through induction of growth factors and cytokines. The growth factors and cytokines induced by these compositions in turn induce a new or enhanced immune response against the tumor cells.

The present invention provides a composition and method for enhancing wound healing by inducing monocytes of a human immunological system to release IL-1, IL-6 and tumor necrosis factor which in turn induces fibrosis in the area of composition application.

Brief Description of the Drawings

FIGURE 1 is a graph showing the induction of TNF by Poly M, het rologous GMGM and Poly G alginates.

FIGURE 2 is a graph showing the dampening effect of induction of TNF by Poly M when combined with Poly G alginate.

FIGURE 3 is a graph showing the induction of IL-1 by Poly M, heterologous GMGM polymeric and Poly G alginates.

FIGURE 4 is a graph showing the dampening effect of induction of IL-1 by Poly M when combined with Poly G alginate.

FIGURE 5 is a graph showing the induction of IL-6 by Poly M, heterologous GMGM and Poly G alginates.

FIGURE 6 is a graph showing the dampening effect of induction of IL-6 by Poly M when combined with Poly G.

Detailed Description of the Invention

The present invention is a composition and a method for enhancing wound healing. The composition is a biopolymer such as alginate or alginic acid, and particularly biopolymers rich in homopolymeric blocks of mannuronic acid or mannuronate (M). The invention also comprises a topical composition containing Poly M or high M biopolymers and methods of using the same.

The biopolymers of the present invention contain at least 70% M residues and preferably contain more than 70% M residues. Alginate so comprised is shown herein to elicit a high response from monocytes in vitro in the production of tumor necrosis factor (TNF), IL-1 and IL-6.

Michaeli teaches, in the '024 patent, the use of collagen with the addition of low levels of "glycosaminoglycan" for wound healing. It should be noted that alginates are not glycosaminoglycans, even though Michaeli refers to them as such. Michaeli's teaching is very different from that in this application: A major difference lies in the use by Michaeli of collagen in the composition. The '024 patent describes a very limited use of materials such as alginates, stating that the collagen concentration should be around 25-35 times greater than the "glycosaminoglycan" concentration. This is directly

opposite to the present application, which does not include any collagen. In fact the present application suggests the use of 100% biopolymer with at least 70% M content.

Additionally, while Michaeli discusses the use of alginate for wound healing, he refers to alginate in general, without any indication that alginate of a particular M content might be preferred over a different M content. Without such an indication, it would be expected that one of average skill in the art would use any of the commercially available alginates. As noted above, these alginates have in the range of 30 to 65% M content. Thus, there is no teaching in Michaeli to use any alginate with M content higher than 65%. There is no teaching that special efforts to obtain alginates or other biopolymers with at least 70% M content would be advantageous for the purpose of wound healing.

The present invention is also a composition and a method for treatment of tumors. Biopolymers having at least 70% molar M content are disclosed herein to induce cytokine release. The cytokines in turn, when induced at the site of tumor antigens, are known to stimulate the immune response against those tumor antigens. The alginate can be applied topically or internally, such as by i.p. or i.v. injection, to the site of the tumor. Additional cytokines or other immune stimulators can be incorporated in the biopolymer composition to further enhance the immune response.

Alternatively, tumor infiltrating cells can be coated and/or bound to biopolymers of this invention and administered to the patient with the tumor. See, for example, Yron et al. (1980) J. Immunol. 125:238; Lotze et al. (1981) Cancer Res. 41:4420; Grimm et al. (1982) J. Exp. Med. 155:1823; and Rosenstein t al. (1984) Cancer Res. 44:1946, where the isolation and growth of tumor infiltrating lymphoid cells are described. These cells will then carry the biopolymers to the site of the tumor, where

the biopolymers will induce cytokine production, with the resulting induction or enhancement of the immune response against the tumor. As with direct application of the biopolymer composition to the tumor, additional cytokines or other immune stimulators can be incorporated in the biopolymer composition to further enhance the immune response.

The biopolymers of this invention can be prepared in a variety of manners. Alginate can be obtained commercially as described above from sources such as Protan. Likewise, alginate can be extracted from biological sources described above, especially from Azotobacter and Pseudomonad species.

Growth factors and cytokines can be obtained for incorporation in this invention from numerous sources. For example, fibroblast growth factor, platelet-derived growth factor, epidermal growth factor, transforming growth factor-α, insulin-like growth factor-I, interleukin-2, interleukin-3, and interleukin-6, all human recombinant, are available from Gibco BRL, and others such as insulin-like growth factor-II and tumor necrosis factor-α are available from Promega.

Biopolymers such as alginate can be used in any form appropriate for covering a wound. For example, for an external wound, alginate or other biopolymer can be made into fibers by methods known in the art and woven, spun mixed or otherwise incorporated into gauze or other common types of dressings. Alternatively, the biopolymer can be in the form of a gel to be spread on the wound or a film to be placed on the wound. It may be injected into a wounded area with an appropriate carrier or inert ingredients. It may be made into a solution at an effective concentration and applied directly to the injured area. The solution may also be applied to dressings covering the wounded area. The biopolymers may also be employed in the form of a powdered fiber.

For internal wounds such as ulcers, the biopolymer can be in the form of a solution to be taken orally that will coat the walls of the gastrointestinal tract. For other internal wounds, the biopolymer can be in a solution or gel that is injected to the site of the wound. Carriers such as gels, ointments, lotions, aqueous solutions and other pharmaceutically acceptable carriers known in the art may be used.

The biopolymers of the present invention may be formulated with conventional pharmaceutical or veterinary aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc. Thus, for internal use, the compounds of the present invention may be in conventional pharmaceutical administration forms such as tablets, capsules, powders, solutions, suspensions, dispersions, syrups, suppositories etc.; however, solutions, suspensions and dispersions in physiologically acceptable carrier media, for example water, will generally be preferred.

The compounds according to the invention may therefore be formulated for administration using physiologically acceptable carriers or excipients in a manner fully within the skill of the art. For example, the compounds, optionally with the addition of pharmaceutically acceptable excipients, may be suspended or dissolved in an aqueous medium, with the resulting solution or suspension then optionally being sterilized. Suitable additives include, for example, physiologically biocompatible buffers.

If the biopolymers are to be formulated in suspension form, e.g., in water or physiological saline, for oral administration, the biopolymer may be mixed with one or more of the inactive ingredients traditionally present in oral solutions and/or surfactants and/or aromatics for flavoring.

Parenterally administrable forms, e.g., intravenous solutions, should be sterile and free from physiologically

unacceptable agents, and should have low osmolality to minimize irritation or other adverse effects upon administration, and thus solutions containing biopolymers should preferably be isotonic or slightly hypertonic. Suitable vehicles include aqueous vehicles customarily used for administering parenteral solutions such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other solutions such as are described in Remington's Pharmaceutical Sciences, 15th ed., Easton: Mack Publishing Co., pp. 1405-1412 and 1461-1487 (1975) and The National Formulary XIV, 14th ed. Washington: American Pharmaceutical Association (1975). The solutions can contain preservatives, antimicrobial agents, buffers and antioxidants conventionally used for parenteral solutions, excipients and other additives which are compatible with the biopolymers and which will not interfere with the manufacture, storage or use of products.

Other various compositions may also be added to the High M or poly M biopolymers of the present invention. For example, growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and transforming growth factor-beta (TGF-β) may be added alone or in combination to a treatment solution or a dressing comprising high M or poly M alginate.

Biopolymers of this invention are shown herein to induce release of certain cytokines. As shown in Figures 1-6, three separate alginate compositions were tested for their ability to induce monocytes to lease TNF, IL-1 and IL-6. The alginate compositions included Poly G alginate, heterologous GMGM alginate comprising linear binary copolymers of 1-4 linked β-D-mannuronic acid (M) and its C-5 pim r α-L-guluronic acid (G) and Poly M (β-D-mannuronic acid) alginate and are referenced in Figures 1-6 as Poly G, GMGM and Poly M.

Example 1

Alginate Preparation

Alginate was prepared for comparison of the effects of various compositions as stimulants for wound healing.

Commercial alginate from the algae Laminaria hyperborea (LF 10/60, batch nr. BL 5417368) containing 64% guluronic acid residues was obtained from Protan A/S, Drammen, Norway. LPS contamination in the alginate was removed by the method described by Karplus et al. ("A new method for reduction of endotoxin contamination from protein solutions"; J. Immunol. Methods, 1987: 105: 211) using a combination of Polymyxin-B-sepharose 4B (PB-Seph 4B) (Pharmacia, Uppsala, Sweden) affinity binding and endotoxin-protein dissociation with the dialyzable sufactant octyl-b-D-glucopyranoside OBDG, Sigma, St. Louis, MO, USA).

Briefly, 1% (w/v) OBDG was added to 1% (w/v) LF 10/60 solution (dissolved in elution buffer consisting of $NaHCO_3$ pH 8.5), and mixed for 30 min. at room temperature. Equal volumes of the PB-Seph 48-gel and OBDG/alginate solution were mixed and transferred to a dialysis bag (MW 12-14000). The bag was then placed in a container with phosphate buffer saline (PBS) and dialyzed for 48 hours at room temperature. Subsequently, the PB-Seph 4B was removed by centrifugation at 2750 r.p.m., for 10 min. at 4°C. 0.2% NaCl (w/v) was added to the alginate solution and the alginate was precipitated with 96% ethanol. The alginate was then washed twice with 96% ethanol and finally once with 96% ethanol and once with diethylether before it was dried. This alginate is referred to herein as poly-G alginate or G-block alginate.

M-blocks alginate (95% M and degree of polymerization (DPn = 35) was obtained from an alginate enriched mannuronic acid isolated from the intracellular substance of Ascophyllum nodosum (A.nodosum) fruiting bodies as described by Haug et al. ("Correlation between chemical structure and physical properties of alginates" Acta chem

scand 1967:21:768). Alginate fragments containing more than 85% of G units and DP_n=40 (G-blocks) were prepared from Laminaria digitata. Alginate fragments with predominantly an alternating structure, MG-blocks (63% M and DP_n=25) were isolated from A. nodosum by the method described by Haug et al. ("Studies on the sekvens of uronic acid residues in alginic acid Acta chem scand 1967:21:691).

An alginate sample with a lower content of guluronic acid residues (46%) was isolated from tissues of A. nodosum as described by Haug et al.

The monomer composition and sequential arrangement as well as the DPn were analyzed by ^{1}H-n.m.r. spectroscopy on a Bruker WM-400 spectrometer as described previously by Grasdalen et al. ("A p.m.r. studie of composition and sequence of uronate residues in alginate"; Carbohydr Res 1979; 68:23)

Endotoxin content in the purified and unpurified alginates was quantified by the LAL-assay (Coatest Endotoxin from Kabi Vitrum, Stockholm, Sweden).

Example 2

Assay for detection of TNF-α in supernatants from monocytes

TNF-α was determined by its cytotoxic effect on the fibrosarcoma cell line WEHI 164 clone 13, as described in Espevik et al. ("A highly sensitive cell line, WEHI 163 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes." J. Immunol Methods 1986; 95:99.) Dilutions of recombinant (r) TNF-α (r-TNF-α, Genentech, Inc. South San Francisco) were included as a standard. The TNF-α specificity of the assay was verified by a monoclonal antibody against rTNF-α which completely neutralized the recorded activity (data not shown).

Example 3

Assay for Detection of IL-1 in supernatants from monocytes

IL-1 was determined by a two stage assay. The first stage involves the mouse thymocyte EL-4 NOB-I cell line which produces high concentrations of IL-2 (interleukin-2) in response to human IL-1, as described by Gaering et al. Dilutions of r-IL-1 (Glaxo, Geneva, Switzerland) were included as standard. After incubation in CO_2 for 24 hours, 100 µl of the supernatants were transferred into replicate 96-well microplates. The second stage in this assay involves the IL-2 dependent mouse T cell line HT-2 as described by Mosmann, T. ("Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays." J. Immunol 1987; 139:4116) One hundred µl of HT-2 suspension (1.2 x 10^5 cells/ml were added to each well and incubated for an additional 24 hours. The IL-1 activity was completely neutralized by two polyclonal antibodies against rIL-1b. Results are presented as pg/ml +/- S.E. of triplicated determinations.

As shown in Figure 1, three separate alginate compositions were tested for their ability to induce monocytes to release TNF. The alginate compositions included Poly G alginate, heterologous GMGM alginate comprising linear binary copolymers of 1-4 linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G) and Poly M (β-D-mannuronic acid) alginate. The foregoing three types of alginate material are referenced in Figures 1 through 6 as Poly G, GMGM and Poly M. The alginates were dissolved in tissue culture medium in varying concentrations set forth in Figure 1, 3 and 5 in which equal concentrations of monocytes were placed. Figure 1 shows that Poly M and GMGM alginate induced substantial TNF production by the monocytes on the order of 7000 to 10,000 picograms of TNF per milliliter, whereas Poly G alginate induced TNF production two orders of magnitude less, or at approximately 200 pg/ml of TNF. TNF is known as a inducer of fibroblast

growth and angiogenesis. Figure 3 shows the equivalent results with respect to IL-I production by the monocytes. Figure 5 shows the equivalent results with respect to IL-6 production.

As shown in Figure 2, Poly G apparently inhibits the production of TNF by monocytes. Figure 2 shows the results of an experiment in which Poly M and Poly M + 1 mg/ml of Poly G was added to a culture of monocytes and the TNF production was measured. As can be seen from the graph, the Poly M + Poly G sample induced substantially lower TNF production than Poly M alone. Thus it appears that Poly G not only has very limited TNF induction capability, it also inhibits Poly M alginate's ability to induce TNF production of monocytes, and accordingly, would inhibit Poly M alginate induction of fibrosis. Figure 4 shows the equivalent results with respect to IL-1 production by the monocytes. Figure 6 shows the equivalent results with respect to IL-6 release.

Viability in the assays for TNF-α, IL-1 and IL-6 were measured in a colorimetric assay for growth and survival by using a tetrazolium salt as described by Mosmann.

Table 1

CYTOKINE RELEASE FROM MONOCYTES CULTURED ON ALGINATE GELS

Treatment	TNF-α (pg/ml)	IL-6 (pg/ml)	IL-1 (pg/ml)
LF 10/60 alginate gel	7000 ±1100	10900 ±1600	6400 ±100
A. nodosum alginate gel	15600 ±5300	15200 ±2000	16300 ±800
1 μg/ml LPS	12400 ±2600	22200 ±5100	9600 ±900
Growth Method	50 ±10	70 ±20	90 ±10

Table 1 shows the results of an experiment which demonstrates cytokine release from monocytes cultured on alginate gels. Monocytes on tissue culture plates were

detached by a rubber policeman, washed once in Hanks Balanced Salt Solution, and added culture wells with alginate gel, or culture wells with LPS or growth media. Alginate gels were made as described above. Supernatants were harvested after 16-24 hours and assayed for TNF, IL-6 and IL-1. As can be seen from the table, the monocytes cultured on LF 10/60, which has a 64% G residue content, induced substantially less production of each of TNF, IL-1 and IL-6 compared with A. nodosum alginate gel, which has a G residue content of 46%. LPS also showed a great capacity to induce cytokine production.

As a result of these findings, the present invention comprises the use of Poly M alginate or high M alginate as an active ingredient in wound healing preparations. Poly M alginate and high M alginate may be obtained from Protan (Norway), or may be obtained by isolation of the material or by chemical conversion by methods reported in the literature. Alginate derived from Azotobacter vinelandii, Pseudomonas aeruginose, P. putida, P. mendocina, P. fluorescens and P. syringae are preferred as providing alginate having a high M content. In fact, the alginate derived from P. syringae has an M content of substantially 100% and has been found to be a potent cytokine inducer.

It will be obvious to a person of ordinary skill in the art that the present invention is not limited in its application to specific alginate compositions disclosed herein. The only limitations of the present invention are

set forth in the claims appended hereto and any equivalents thereof.

Claims

1. A method of treating wounds comprising applying to a wound a biopolymer composition, said composition comprising at least 70% molar β-D-mannuronic acid and β-D-mannuronate.

2. The method of claim 1 wherein the composition is selected from alginic acid, alginate, and a combination of alginic acid and alginate.

3. The method of claim 1 wherein said composition also comprises α-L-guluronic acid and α-L-guluronate.

4. The method of claim 1 wherein the remainder of said composition is selected from α-L-guluronic acid and α-L-guluronate.

5. The method of claim 1 wherein the β-D-mannuronic acid and β-D-mannuronate are in polymers having molecular weight more than 10,000 daltons.

6. The method of claim 5 wherein the molecular weight of the polymers is at least 50,000 daltons.

7. The method of claim 1 further comprising applying to said wound a growth factor selected from epidermal growth factor, fibroblast growth factor, platelet derived growth factor and transforming growth factor-beta.

8. The method of claim 1 further comprising applying to said wound a cytokine selected from interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5 and interleukin-6.

9. The method of one of claims 1-8 comprising incorporating said composition into a wound dressing and applying said wound dressing to said wound.

10. A method of making a composition useful as a wound healing agent, comprising providing a biopolymer composition comprising at least 70% β-D-mannuronic acid and β-D-mannuronate, the remainder comprising α-L-guluronic acid and α-L-guluronate, and mixing said composition with a pharmaceutically acceptable carrier material.

11. A composition capable of inducing fibrosis to a wound, said composition comprising an biopolymer containing at least 70% β-D-mannuronic acid and β-D-mannuronate.

12. The composition of claim 11 wherein the composition is selected from alginic acid, alginate, and a combination of alginic acid and alginate.

13. The composition of claim 11 wherein said composition also comprises α-L-guluronic acid and α-L-guluronate.

14. The composition of claim 11 wherein the remainder of said composition is selected from α-L-guluronic acid and α-L-guluronate.

15. The composition of claim 11 wherein the β-D-mannuronic acid and β-D-mannuronate are in polymers having molecular weight more than 10,000 daltons.

16. The composition of claim 15 wherein the molecular weight of the polymers is at least 50,000 daltons.

17. The composition of claim 11 further comprising a growth factor selected from epidermal growth factor, fibroblast growth factor, platelet derived growth factor and transforming growth factor-beta.

18. The composition of claim 11 further comprising a cytokine selected from interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5 and interleukin-6.

19. The composition of claim 11 wherein said composition is in gel form.

20. The composition of claim 11 wherein said composition is in an ointment.

21. The composition of claim 11 wherein said composition is in powder form.

22. The composition of claim 11 wherein said composition is in fiber form.

23. The composition of claim 22 wherein said composition is in the form of a powdered fiber.

24. The composition of claim 11 wherein said composition is incorporated into a gauze-like material.

25. The composition of claim 11 wherein said composition is in the form of a film.

26. The composition of claim 11 wherein said composition is in the form of a shaped object.

27. The composition of claim 26 wherein said shaped object is a bead.

28. A method of tumor therapy comprising applying to a tumor a biopolymer composition, said composition

comprising at least 70% molar β-D-mannuronic acid and β-D-mannuronate.

29. The method of claim 28 wherein the composition is selected from alginic acid, alginate, and a combination of alginic acid and alginate.

30. The method of claim 28 wherein said composition also comprises α-L-guluronic acid and α-L-guluronate.

31. The method of claim 28 wherein the remainder of said composition is selected from α-L-guluronic acid and α-L-guluronate.

32. The method of claim 28 wherein the β-D-mannuronic acid and β-D-mannuronate are in polymers having molecular weight more than 10,000 daltons.

33. The method of claim 32 wherein the molecular weight of the polymers is at least 50,000 daltons.

34. The method of claim 28 further comprising applying to said tumor a cytokine selected from interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5 and interleukin-6.

35. A composition capable of enhancing the immune response to a tumor, said composition comprising a biopolymer containing at least 70% β-D-mannuronic acid and β-D-mannuronate.

36. The composition of claim 35 wherein the composition is selected from alginic acid, alginate, and a combination of alginic acid and alginate.

37. The composition of claim 35 wherein said composition also comprises α-L-guluronic acid and α-L-guluronate.

38. The composition of claim 35 wherein the remainder of said composition is selected from α-L-guluronic acid and α-L-guluronate.

39. The composition of claim 35 wherein the β-D-mannuronic acid and β-D-mannuronate are in polymers having molecular weight more than 10,000 daltons.

40. The composition of claim 39 wherein the molecular weight of the polymers is at least 50,000 daltons.

41. The composition of claim 35 further comprising a cytokine selected from interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5 and interleukin-6.

42. The composition of claim 35 wherein said composition is in gel form.

43. The composition of claim 35 wherein said composition is in an ointment.

44. The composition of claim 35 wherein said composition is in powder form.

45. The composition of claim 35 wherein said composition is in fiber form.

46. The composition of claim 45 wherein said composition is in the form of a powdered fiber.

47. The composition of claim 35 wherein said composition is incorporated into a gauze-like material.

48. The composition of claim 35 wherein said composition is in the form of a film.

49. The composition of claim 35 wherein said composition is in the form of a shaped object.

50. The composition of claim 49 wherein the shaped object is a bead.

51. A method of tumor therapy comprising attaching to tumor infiltrating cells a biopolymer composition, said composition comprising at least 70% molar β-D-mannuronic acid and β-D-mannuronate.

52. The method of claim 51 wherein the means of attaching is by coating the cells with the biopolymer composition.

53. The method of claim 51 wherein the composition is selected from alginic acid, alginate, and a combination of alginic acid and alginate.

54. The method of claim 51 wherein said composition also comprises α-L-guluronic acid and α-L-guluronate.

55. The method of claim 51 wherein the remainder of said composition is selected from α-L-guluronic acid and α-L-guluronate.

56. The method of claim 51 wherein the β-D-mannuronic acid and β-D-mannuronate are in polymers having molecular weight more than 10,000 daltons.

57. The meth d of claim 56 wherein the molecular weight of the polymers is at least 50,000 daltons.

58. The method of claim 51 further comprising attaching to said tumor infiltrating cells a cytokine

selected from interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5 and interleukin-6.

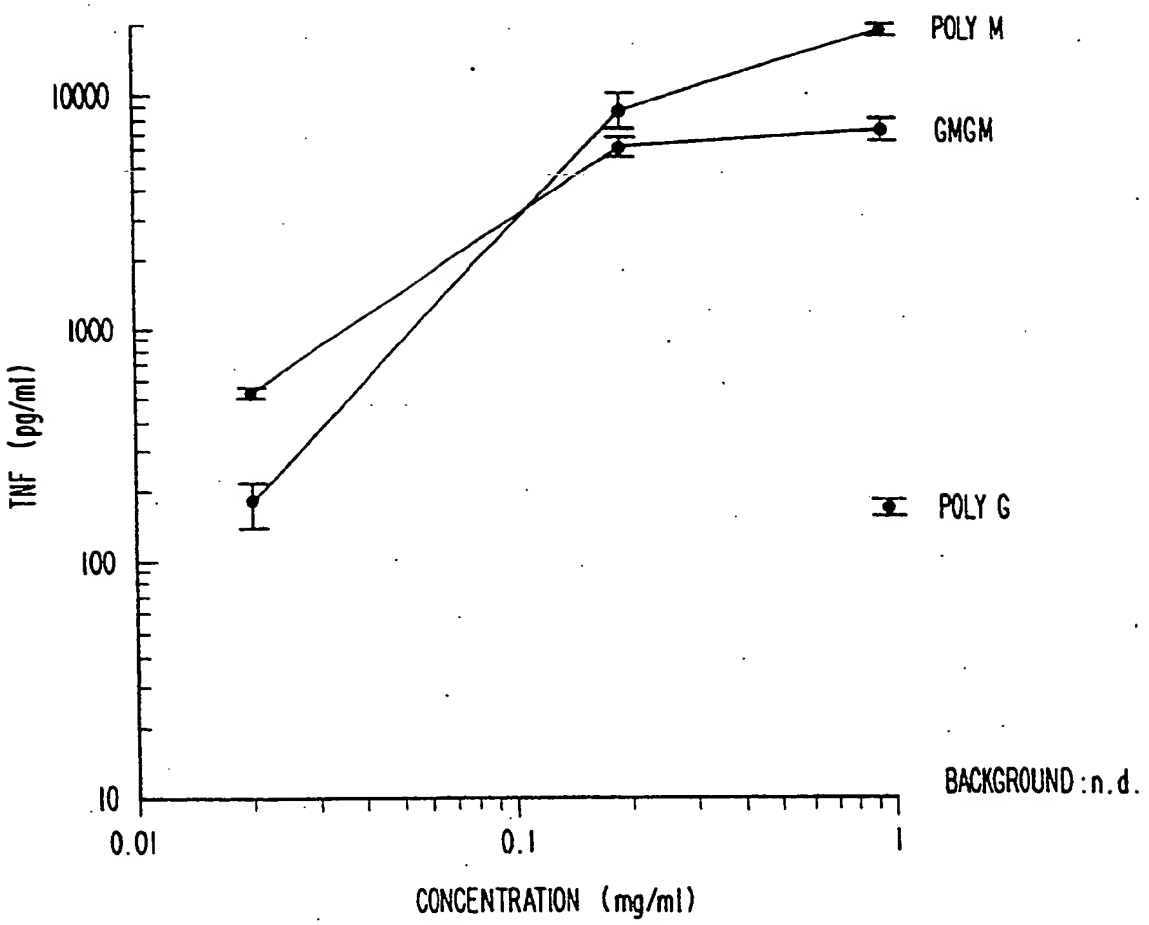
POLY M
GMGM
POLY G
BACKGROUND : n.d.
TNF (pg/ml)
10000
1000
100
10
0.01
0.1
1
CONCENTRATION (mg/ml)

FIG. 1.

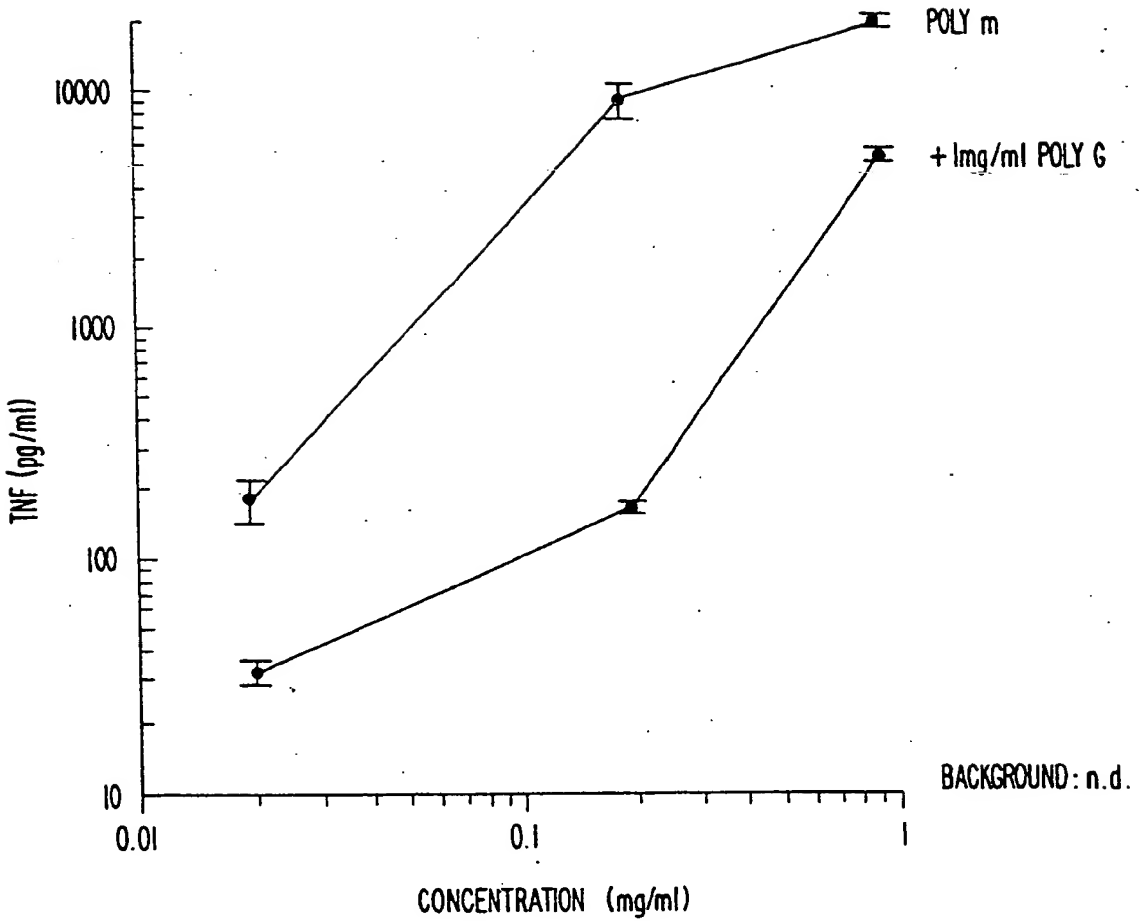
POLY m
+ Img/ml POLY G
BACKGROUND: n.d.
TNF (pg/ml)
10000
1000
100
10
0.01
0.1
1
CONCENTRATION (mg/ml)

FIG. 2.

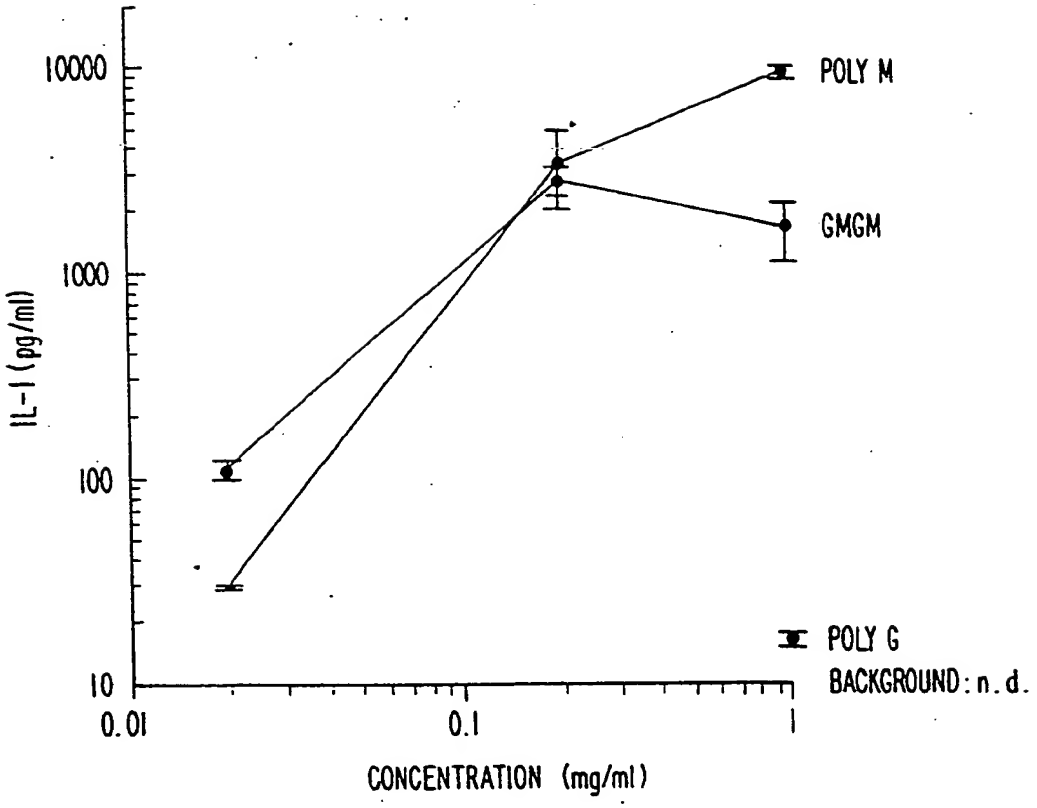
10000
1000
100
10
IL-1 (pg/ml)
0.01
0.1
1
CONCENTRATION (mg/ml)
POLY M
GMGM
POLY G
BACKGROUND: n.d.

FIG. 3.

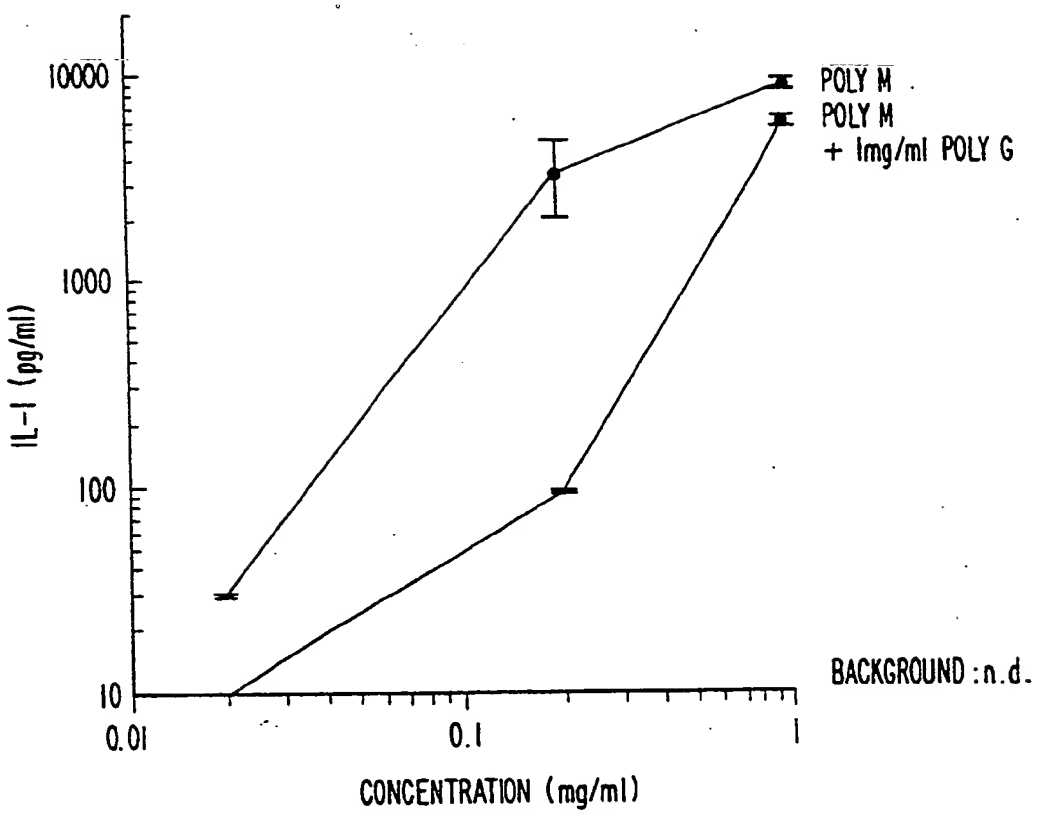
10000
1000
100
10
IL-1 (pg/ml)
0.01
0.1
1
CONCENTRATION (mg/ml)
POLY M
POLY M
+ 1mg/ml POLY G
BACKGROUND : n.d.

FIG. 4.

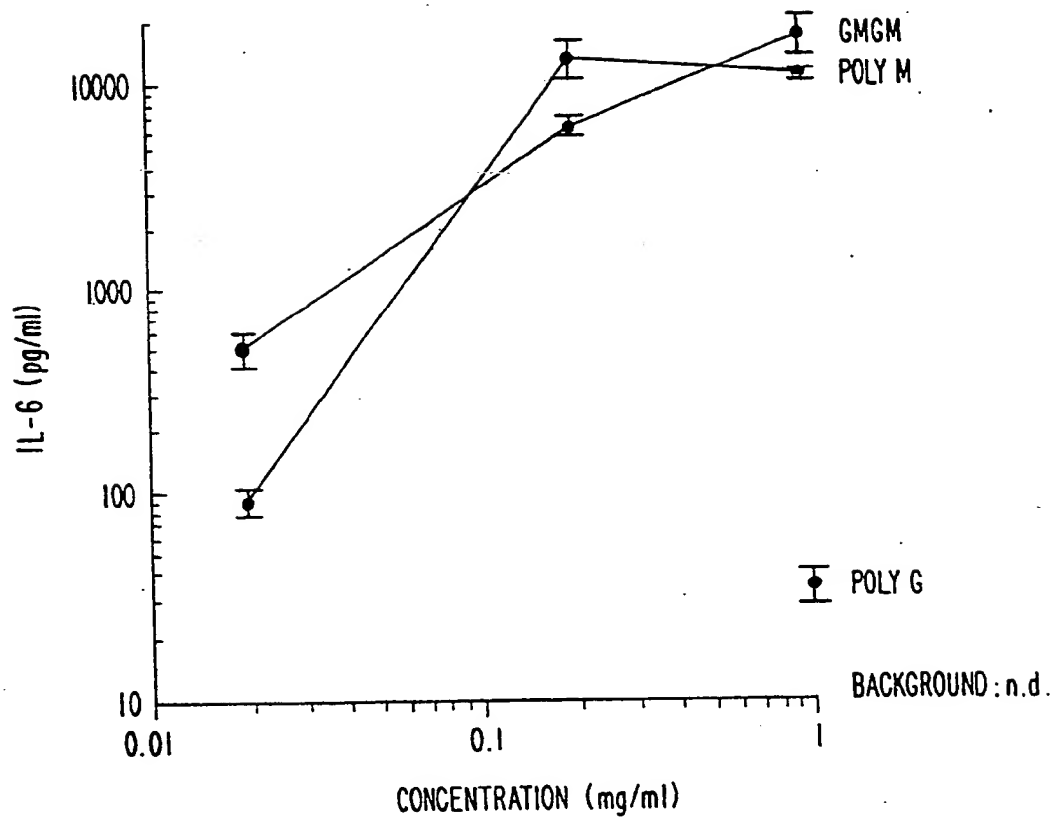
GMGM
POLY M
POLY G
BACKGROUND : n.d.
IL-6 (pg/ml)
10000
1000
100
10
0.01
0.1
1
CONCENTRATION (mg/ml)

FIG. 5.

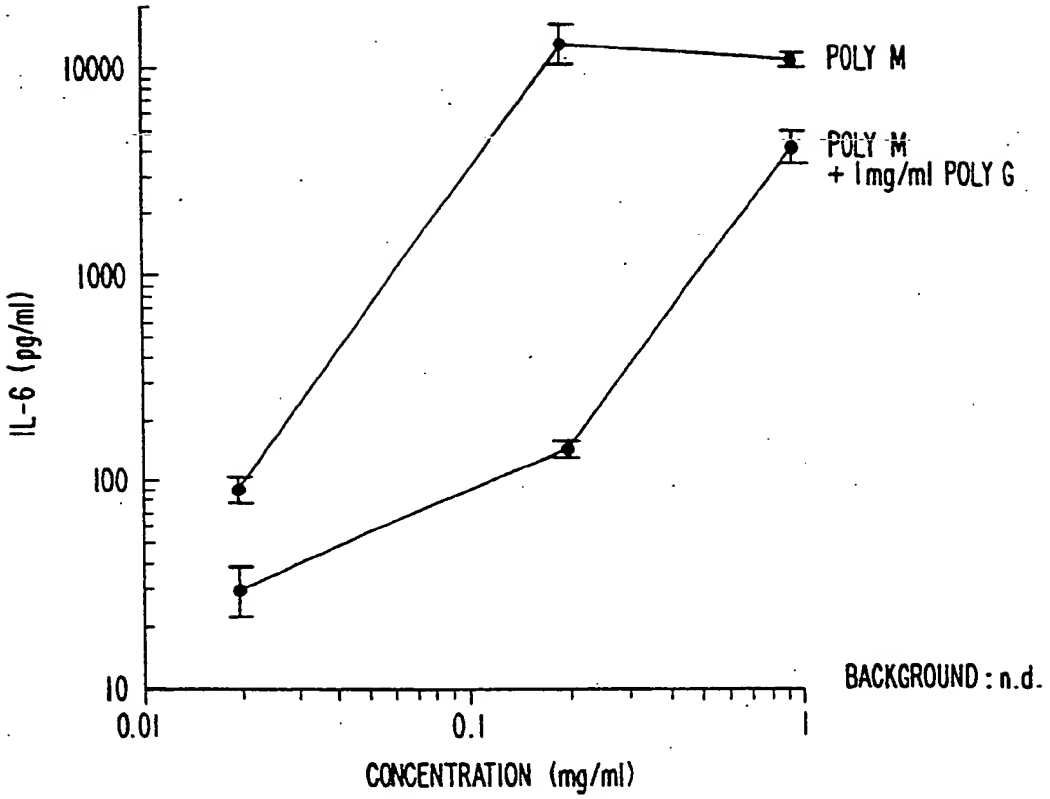
10000
1000
100
10
IL-6 (pg/ml)
0.01
0.1
1
CONCENTRATION (mg/ml)
POLY M
POLY M
+ 1mg/ml POLY G
BACKGROUND : n.d.

FIG. 6.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/00475

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61L 15/00; A61K 37/02,37/36
U.S.CL: 424/445,85.1,85.2; 514/2,12,21,54,62

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
U.S.	424/445, 449, 69, 78, 85.1,85.2; 514/2,12,21,54,62

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

-DIALOG: B Biochem (one search category)

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	US, A, 4,837,024 (MICHAELI) 06 June 1989 See column 10, lines 15-53.	1-58
Y	US, A, 4,619,913 (LUCK) 28 October 1986 See column 5, line 7.	1-58

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 April 1991	21 JUN 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Leon Horne

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1-27 drawn to wound healing, composition for wound healing, method of making a dressing and method of making a composition, class 424, subclass 78.

see attached sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

telephone practice

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4 ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.